



Original Article

Antinociceptive and anti-inflammatory effects of the citrus flavanone naringenin

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ABSTRACT

Objective: Naringenin, a flavonoid found in citrus fruits, has notably diverse pharmacological properties. In the present study, we investigated the antinociceptive and anti-inflammatory effects of naringenin. **Materials and Methods:** The antinociceptive effects were evaluated using hot-plate, acetic acid-induced writhing, and tail-flick assays in mice and rats. The anti-inflammatory effects were examined by a carrageenan-induced paw edema test in rats. **Results:** Naringenin (100 or 200 mg/kg, oral administration) significantly delayed the reaction time of mice to thermal stimulation generated by a hot plate and a tail-flick unit and reduced the acetic acid-induced writhing response in mice. In addition, naringenin significantly decreased paw edema induced by carrageenan in rats, showing its anti-inflammatory effect. **Conclusion:** Our results show that naringenin has therapeutic potential with antinociceptive and anti-inflammatory properties and can further be exploited for the development of drugs for pain and inflammatory-related diseases.

KEYWORDS: *Anti-inflammatory, Antinociceptive, Inflammation, Naringenin, Pain*

INTRODUCTION

Pain is one of the general manifestations of inflammation, as inflammatory cytokines and mediators sensitize primary afferent neurons to subsequent stimuli. Specific medical treatment is critical in dealing with it properly [1]. To understand the concept of pain, it is important to recognize that pain which occurs after various noxious stimuli is often protective and quite distinct from the pain caused by significant damage to tissues or nerves [2]. The main characteristic of inflammatory states is that usually harmless stimuli produce pain [3,4].

At present, most anti-inflammatory drugs are effective in inhibiting inflammation, but have many serious side effects such as stomach ulcers, gastrointestinal bleeding, and cardiovascular effects. These limitations have driven drug researches to develop new pain therapies [5-7]. Natural products and their derivatives have been recognized for many years as sources for new therapeutic agents, as new treatments are considered

important in overcoming the existing adverse effects in the treatment of pain [8,9].

Naringenin, an aglycone of naringin, is a predominant flavanone glycoside found in grapes and citrus fruits [Figure 1]. Orally administered naringin needs to be hydrolyzed by intestinal bacteria to naringenin before being absorbed [10]. Naringenin has many pharmacological properties such as antidiabetic, antiobesity, antidiabetic, antimicrobial, and anticancer effects [11-15]. Recently, it was also found to have potential for immunomodulatory efficacy, which could be associated with suppression of the activities of mast cells,

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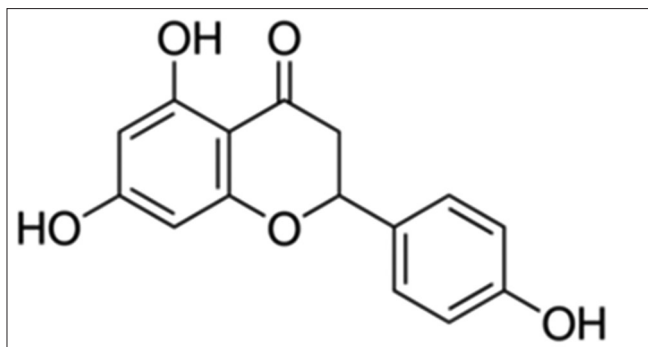


Figure 1: Chemical structure of naringenin

macrophages, dendritic cells, and T cells [16-20]. Therefore, naringenin has been proposed for potential pharmaceutical application in rheumatoid arthritis and osteoarthritis [19,21,22].

Although these findings demonstrate the anti-inflammatory potential of naringenin, little is known about its possible antinociceptive activity. Hence, in the present study, we further investigated the antinociceptive activity of naringenin in experimental models.

MATERIALS AND METHODS

Animals

Male BALB/c mice (20 ± 2 g) and male Sprague-Dawley rats (150–160 g) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). The mice were fed sterilized mouse chow and water. All animal experiments were conducted in accordance with the Laboratory Animal Management and Ethics Committee of National Chung Hsing University. Animals were randomly divided into five groups. Animals in Group I were treated with corn oil (Sigma-Aldrich, St. Louis, MO, USA) as the vehicle control. Animals in Groups II, III, and IV were orally administered 50, 100, and 200 mg/kg naringenin dissolved in corn oil (Sigma-Aldrich, St. Louis, MO, USA), respectively, using oral gavage needles. Animals in Group V received standard pain-relieving drugs, such as codeine, aspirin, or ibuprofen, as positive controls, where indicated. All experiments were approved according to the guidelines of the Laboratory Animal Care and Use Committee of National Chung Hsing University, Taiwan.

Hot-plate test in mice

Animals were administered naringenin orally once daily for 1 or 2 weeks before the hot-plate test and abdominal writhing test. The mouse was placed on a hot-plate apparatus (UGO, Basile, Italy) with a temperature of $55^{\circ}\text{C} \pm 1^{\circ}\text{C}$, and the latency was measured. The latency is an index of the pain threshold, defined as the period of time from when the mouse was placed on the hot plate to the first sign of lifting or licking its hind paws, or jumping from the surface to avoid the heat. The total time would not exceed 60 s to avoid further injury to the animal. Mice which received 10 mg/kg codeine (Sigma/RBI, Natick, MA, USA) were the positive controls [23].

Acetic acid-induced abdominal writhing test in mice

This test has been described previously [24]. Briefly, mice were injected intraperitoneally with 0.7% acetic acid (0.1 mL/10 g) (Merck, Darmstadt, Germany) solution. The numbers of writhing responses in the mice, particularly manifesting as abdominal muscular contractions and hindlimb stretches, were counted during a 20 min period after acetic acid injection. The data express the total number of responses observed over the 20 min. Mice which received 100 mg/kg of aspirin (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally were used as positive controls [23].

Tail-flick test in rats

For the tail-flick and paw edema tests, animals were treated with naringenin once before the respective measurement. In brief, rats were placed on the tail-flick unit (UGO Basile, Italy), and their tails received heat stimuli generated by an infrared laser beam. The nociceptive threshold was recorded as the time from laser startup to the time the animal flicked its tail. Rats treated with 10 mg/kg codeine were used as positive controls [23].

Carrageenan-induced edema in rats

This method was utilized as described in a previous study with slight modifications [24]. Carrageenan 1% (Sigma-Aldrich, St. Louis, MO, USA) diluted with normal saline was injected into the right hind paws of rats (100 μL /animal) with or without 1-week naringenin treatment to trigger paw edema. The paw volumes were measured hourly from 1 to 5 h after injection using a plethysmometer (UGO Basile, Italy). Rats given 100 mg/kg ibuprofen (Sigma-Aldrich, St. Louis, MO, USA) were the positive controls.

Statistical analyses

Data were presented as mean \pm standard deviation, unless otherwise indicated. The results were determined using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was examined by one-way analysis of variance, or two-way analysis of variance was used to compare multiple groups in the experiments.

RESULTS

Effects of naringenin on thermal nociception in mice

Data on the antinociceptive activity of naringenin in the hot-plate tests are shown in Figure 2. There were no significant differences between the 50 mg/kg naringenin group and vehicle group. Mice treated with 100 and 200 mg/kg naringenin had prolonged response times to the heat of the hot plate. Specifically, the average hot-plate latencies for mice treated with naringenin for 1 week were 11.8 (100 mg/kg), 12.6 (200 mg/kg), and 7.6 (vehicle) s and for those with 2-week treatment were 12.8 (100 mg/kg), 19.4 (200 mg/kg), and 8.0 (vehicle), respectively. However, there was no significant difference between the 1 week and 2 week naringenin treatment groups (two-way ANOVA). The data reveal that naringenin could have antinociceptive effects on thermal nociception manifested as delayed response times.

Effects of naringenin on acetic acid-induced writhing nociception in mice

As shown in Figure 3, oral administration of naringenin (100 and 200 mg/kg) in the test mice significantly reduced

the number of muscular contractions and hindlimb stretches resulting from acetic acid-induced abdominal writhing. Control mice were administered vehicle for 1 and 2 weeks averaged 64.2 and 61.4 stretching responses, respectively, within 20 min. However, treatment with 100 mg/kg naringenin reduced the numbers to 50.6 and 48.6, and 200 mg/kg naringenin decreased the responses to 32.6 and 25.6, compared with 21.8 and 15.2 responses in the positive controls (aspirin administration).

Effects of naringenin on tail-flick test in rats

In the tail-flick tests, the durations of the reaction time (tail-flicking latency) in the 100 and 200 mg/kg naringenin treatment groups were longer than those of mice in the vehicle control group [Figure 4]. Administration of 100 and 200 mg/kg naringenin for both 1 and 2 weeks increased the tail-flicking latency an average 37.8% and 43.9%, respectively, compared with the vehicle control group rats and codeine-treated positive control group, which recorded a 54.1% increase in reaction time.

Effects of naringenin on carrageenan-induced paw edema in rats

The injection of carrageenan into the hind paws of rats was utilized as a model of acute inflammation. The volumes of paws with or without naringenin treatment for 1 week are summarized in Table 1. Consecutive oral administration of 100 mg/kg and 200 mg/kg naringenin for 1 week before the carrageenan injection resulted in a decrease in paw swelling for at least 5 h. The paw volumes were reduced by 16.2% and 37.7%

in the 100 and 200 mg/kg treatment groups, respectively, by 3 h compared with the vehicle control group. Comparably, the standard pain-relieving drug, ibuprofen, used in this test as a positive control treatment, decreased edema by 59.4% at 3 h.

DISCUSSION

In the present study, we evaluated the potential antinociceptive and anti-inflammatory effects of naringenin through multiple pharmacological approaches, including the hot-plate test, acid-induced writhing test, tail-flick test, and carrageenan-induced edema. Our results showed that naringenin had statistically significant antinociceptive and anti-inflammatory effects in treated animals compared with vehicle control animals. To the best of our knowledge, our study is the first analysis to determine if naringenin can alleviate the nociception generated from thermal apparatus in addition to chemical-induced inflammatory pain.

The hot-plate test has been utilized to evaluate the effects of centrally but not peripherally acting analgesics. The tail-flick test is another method to determine acute spinally mediated-nociception to thermal noxious stimuli. In spite of the similar purposes of these methods, it is hard to distinguish whether the pain-relieving perception of rodents resulting from naringenin is due to the peripheral or central nervous system. However, it is noteworthy that the thermal effect of analgesics in the tail-flick pain model is considered to be highly relevant to pain perception in humans [25].

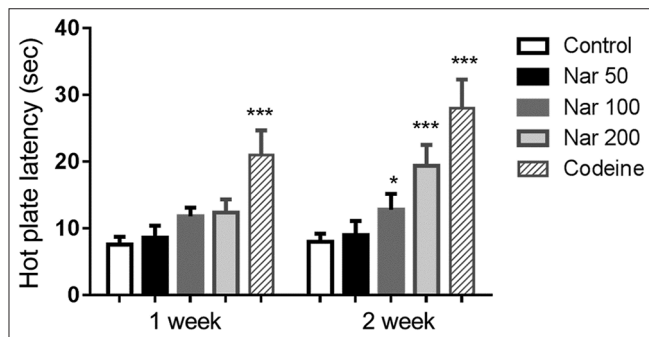


Figure 2: Effects of naringenin on the pain threshold determined by a hot-plate test. Mice were placed on a hot plate at 55 ± 1°C. The latency was defined as the time to the first sign of paw licking or jumping response. Control, corn oil-treated vehicle group; Nar 50, mice treated with naringenin 50 mg/kg; Nar 100, treated with naringenin 100 mg/kg; Nar 200, treated with naringenin 200 mg/kg; Codeine, mice given 10 mg/kg codeine used as positive controls. Data are expressed as the mean ± standard deviation of three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 (two-way ANOVA) compared with the vehicle control group

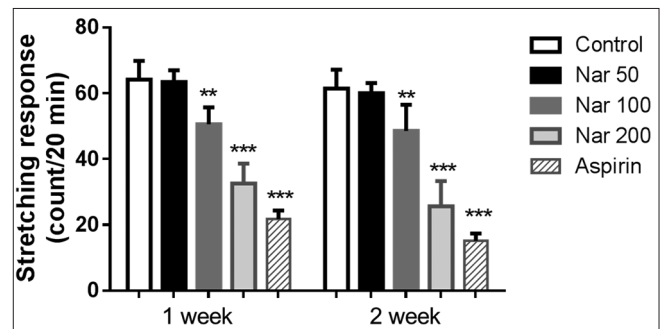


Figure 3: Effects of naringenin on acetic acid-induced writhing responses in mice. The writhes were induced by intraperitoneal injection of 0.7% acetic acid in saline solution. The numbers of muscular contractions and hindlimb stretches of mice were counted for 20 min after injection. Data are expressed as the mean ± standard deviation of three independent experiments. Control, corn oil-treated vehicle group; Nar 50, mice treated with naringenin 50 mg/kg; Nar 100, treated with naringenin 100 mg/kg; Nar 200, treated with naringenin 200 mg/kg; Aspirin, mice treated with aspirin 100 mg/kg used as positive controls. *P < 0.05; **P < 0.01; ***P < 0.001 (two-way ANOVA) compared with the vehicle control group

Table 1: Effects of naringenin in the carrageenan-induced paw edema test

Group	Swelling thickness (×10 ⁻³ mL)				
	1 h	2 h	3 h	4 h	5 h
Control	1.9±0.05	2.8±0.04	3.7±0.05	2.7±0.05	2.1±0.06
Nar 50	1.9±0.07	2.7±0.05	3.6±0.09	2.6±0.09	2.1±0.06
Nar 100	1.7±0.14***	2.3±0.03***	3.1±0.05***	2.3±0.04***	1.9±0.03***
Nar 200	1.2±0.03***	2.2±0.04***	2.3±0.05***	2.1±0.06***	1.8±0.04***
Ibuprofen	0.09±0.02***	1.3±0.04***	1.5±0.03***	1.2±0.04***	1.2±0.02***

Data are expressed as the mean±SD of three independent experiments. ***P<0.001 (two-way ANOVA) compared with the vehicle control group. Ibuprofen (100 mg/kg body weight) was used in the positive control group. Control: Corn oil-treated vehicle group, Nar 50: Mice treated with naringenin 50 mg/kg, Nar 100: Treated with naringenin 100 mg/kg, Nar 200: Treated with naringenin 200 mg/kg, SD: Standard deviation, ANOVA: Analysis of variance

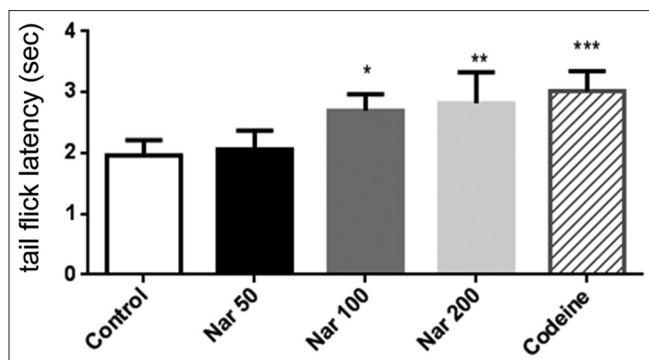


Figure 4: Effects of naringenin on tail-flick test. Rats were placed over a tail-flick unit where their tails received heat stimuli generated by an infrared laser beam. The time latency was measured until the tail flicked. Data are expressed as the mean \pm standard deviation of three independent experiments. Control, corn oil-treated vehicle group; Nar 50, mice treated with naringenin 50 mg/kg; Nar 100, treated with naringenin 100 mg/kg; Nar 200, treated with naringenin 200 mg/kg; Codeine, mice given 10 mg/kg codeine used as positive controls. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (one-way ANOVA) compared with the vehicle control group

Carrageenan-induced paw edema is commonly used to test the anti-inflammatory and antiedema effects of natural products [26]. The neurological nociception of carrageenan-induced paw edema is believed to be a biphasic event, which means that the sources of pain are from the irritation caused by the injection and from tissues compressed by accumulated edema by the end of the 1st h [27]. It has been shown that the expression of cyclooxygenase (COX), which turns into prostaglandin as a major mediator of pain, can be provoked by carrageenan injection [28]. Given that ibuprofen targets the inhibition of COX, we are convinced that naringenin might have a similar mechanism of action to exert its antinociceptive function. In addition, injections of acetic acid and carrageenan produce peripheral analgesic activity by eliciting bradykinins, prostaglandins, and proinflammatory cytokines such as interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor- α , which then activate the chemosensitive nociceptors that excite the inflammatory cytokines [27-29]. Li *et al.* also demonstrated that naringenin could suppress lipopolysaccharide-induced inflammatory cytokines in our previous report [19]. Anti-inflammatory activity might be one of the mechanisms by which naringenin achieves antinociception.

CONCLUSION

Our results show that naringenin exhibits antinociceptive and anti-inflammatory effects via enforcing the pain tolerance of the neural system *in vivo*, providing a scientific basis for its application for alleviating pain and treating inflammatory disorders. Further detailed investigation is required to elucidate the mechanism of action.

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Conflicts of interest

There are no conflicts of interest.

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